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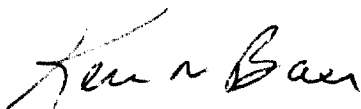
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**§799.5115: Chemical testing requirements for certain chemicals of interest to the  
Occupational Safety and Health Administration  
OPPT-2003-0006**

On behalf of Deltech Corporation, I have enclosed six copies of the final report entitled "Vinyl Toluene: *In Vitro* Dermal Absorption Rate Testing" in compliance with the following test rule: "In Vitro Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration", Federal Register, April 26, 2004 (Volume 69, Number 80), Docket Number OPPT-2003-0006. The study followed the test requirements contained in Title 40, Part 799, Subpart D, §799.5115.

Please direct any questions/comments to me using the contact information below.

Sincerely,



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*Study Title*

Vinyl Toluene:  
*In Vitro* Dermal Absorption Rate Testing

**TEST GUIDELINES:** OECD Guideline for the Testing of Chemicals. Draft New  
Guideline 428: Skin Absorption: *in vitro* Method. (2002)

OECD Draft Guidance Document for the Conduct of Skin  
Absorption Studies. OECD Environmental Health and Safety  
Publications Series on Testing and Assessment No. 28. (2002)

European Commission Guidance Document on Dermal  
Absorption. Sanco/222/2000 rev 6 (2002).

**AUTHOR:** William J. Fasano, Sr., B.S.

**STUDY COMPLETED ON:** January 26, 2005

**PERFORMING LABORATORY:** E.I. du Pont de Nemours and Company  
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Newark, Delaware 19714-0050  
U.S.A.

**LABORATORY PROJECT ID:** DuPont-15743

**WORK REQUEST NUMBER:** 15497

**SERVICE CODE NUMBER:** 1623

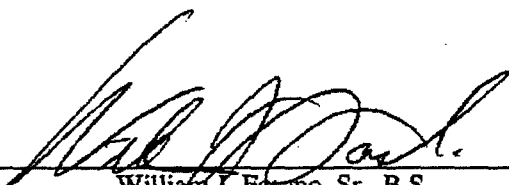
**SPONSOR:** Deltech Corporation  
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Baton Rouge, Louisiana 70807-1318  
U.S.A.

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### GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

This study was conducted in compliance with U.S. EPA TSCA (40 CFR part 792) Good Laboratory Practice Standards, which are compatible with the OECD Principles of Good Laboratory Practice (as revised 1997), ENV/MC/CHEM(98)17, OECD, Paris, 1998, and MAFF Japan Good Laboratory Practice Standards (11 NohSan Number 6283).

Study Director:



William J. Fasano, Sr., B.S.  
Research Toxicologist  
Haskell Laboratory for Health and  
Environmental Sciences

26-JAN-2005

Date

## QUALITY ASSURANCE DOCUMENTATION

Work Request Number: 15497  
Study Code Number: 1623

The conduct of this study has been subjected to periodic Quality Assurance inspections. The dates of inspection are indicated below.

<i>Phase Audited</i>	<i>Audit Dates</i>	<i>Date Reported to Study Director</i>	<i>Date Reported to Management</i>
Conduct:	November 10, 2004 November 11, 2004	November 10, 2004 November 11, 2004	November 10, 2004 November 11, 2004
Report/Records:	January 4, 5, 2005	January 6, 2005	January 17, 2005


Reported by:


  
\_\_\_\_\_  
Joseph C. Hamill  
Quality Assurance Auditor

26-JAN-2005  
Date

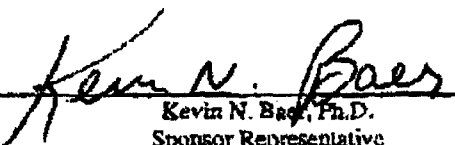
### CERTIFICATION

We, the undersigned, declare that this report provides an accurate evaluation of data obtained from this study.

Approved by:  26-JAN-2005  
Gary W. Jepson, Ph.D.  
Research Manager Date

Issued by Study Director:  26-JAN-2005  
William J. Fasano, Sr., B.S.  
Research Toxicologist Date

This report is approved by the sponsor.

Approved by:  1-25-05  
Kevin N. Baer, Ph.D.  
Sponsor Representative Date

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## STUDY INFORMATION

Substance Tested: Vinyl Toluene

Synonyms/Codes:

- methyl styrene
- ethenylmethylbenzene
- methylvinylbenzene
- methylethenylbenzene
- ar-methyl styrene
- tolyethylene
- meta- and para-vinyl toluene (mixed isomers)

Haskell Number: 26622

CAS Registry Number: 25013-15-4

Composition: Meta- and para-toluene ratio = 1.15

Purity: 99.49%

Known Impurities:

- 0.24 wt% Lights
- 0.10 wt% Heavies
- 0.17 wt% Ethyl toluenes

Physical Characteristics: Colorless liquid

Stability: The test substance appeared to be stable under the conditions of the study; no evidence of instability was observed.

Study Initiated/Completed: September 29, 2004 / (see report cover page)

Experimental Start/Termination: November 10, 2004 / November 15, 2004

## SUMMARY

The permeability coefficient ( $K_p$ ) and the short-term absorption rates at 10 and 60 minutes have been determined for vinyl toluene using human abdominal skin from cadavers mounted in an *in vitro* static diffusion cell model. Human cadaver skin was heat-treated at approximately 60°C and the epidermis was peeled from the dermis and the section mounted onto an *in vitro* static diffusion cell, *stratum corneum* uppermost, with an exposure area of 0.64 cm<sup>2</sup>. Using a recirculating water bath system, the receptor fluid (0.9% saline) was maintained at 32°C. Following system equilibration, skin integrity was confirmed by electrical impedance. The saline in the donor and receptor chambers was removed and discarded and the donor chamber filled with 0.9% saline fortified with 6% polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether).

For the permeability coefficient experiment, an infinite dose of vinyl toluene (100 µL/cm<sup>2</sup>) was applied to the epidermal surface, via the donor chamber, to 6 skin replicates representing 3 human subjects, and the donor chamber opening was occluded with Parafilm®. Serial receptor fluid samples were taken at 1, 2, 4, 8, 12, 24, 36, and 48 hours post-application and analyzed for vinyl toluene by HPLC-UV. At the end of the 48-hour exposure, excess vinyl toluene was removed by washing with a 2% soap solution followed by rinsing with water. The receptor and donor chambers were filled with 0.9% saline and an end of experiment integrity assessment was determined using electrical impedance.

For the short-term exposure experiments, a finite dose of vinyl toluene (10 µL/cm<sup>2</sup>) was applied to the epidermal surface, via the donor chamber, to 12 skin replicates representing 3 human subjects, and the donor chamber opening was occluded with Parafilm®. At the end of the required exposure interval (10 minute and 60 minutes), 6 replicates each were terminated. At termination, the skin surface was washed with a 2% soap solution, rinsed with water, and the receptor fluid was removed and retained for analysis. The receptor and donor chambers were filled with 0.9% saline and end of experiment integrity assessment was taken using electrical impedance. The saline in both chambers was removed and discarded and the skin membrane removed and placed into a glass vial containing methanol. The receptor fluid and the skin (methanol) extract were analyzed for vinyl toluene by HPLC-UV.

Based on the slope at steady-state (203.3 µg/cm<sup>2</sup>/h) and the concentration of the applied dose of vinyl toluene, taken as its density (894,600 µg/cm<sup>3</sup>), the permeability coefficient was calculated to be  $2.27 \times 10^{-4}$  cm/h.

Following a 10-minute exposure to a finite application of vinyl toluene, a total of 3.39 µg of vinyl toluene was detected in the receptor fluid, with nearly an equivalent amount in the skin (3.79 µg). Based on the amount of vinyl toluene in the receptor fluid and skin (7.18 µg), an exposure area of 0.64 cm<sup>2</sup>, and an exposure time of 0.17 hours, the short-term exposure rate was calculated to be 66 µg/cm<sup>2</sup>/h.

Following a 60-minute exposure to a finite application of vinyl toluene, a total of 59.8 µg of vinyl toluene was detected in the receptor fluid and 6.86 µg in the skin. Based on the amount of

vinyl toluene in the receptor fluid and skin (66.7  $\mu\text{g}$ ), an exposure area of 0.64  $\text{cm}^2$ , and an exposure time of one hour, the short-term exposure rate was calculated to be 104.2  $\mu\text{g}/\text{cm}^2/\text{h}$ .

## INTRODUCTION

EPA has promulgated a final rule under the Toxic Substances Control Act (TSCA) that requires manufacturers, importers, and processors of certain chemicals to conduct *in vitro* dermal absorption rate testing. The data obtained under this test rule entitled "*In Vitro* Dermal Absorption Rate Testing of Certain Chemicals of Interest to the Occupational Safety and Health Administration," published in the Federal Register April 26, 2004 (Volume 69, Number 80), will be used by OSHA to evaluate the need for skin designations for the selected chemicals. Skin designations are used to alert industrial hygienists, employers, and workers to the potential contribution of dermal exposure to overall systemic toxicity.

The objective of this study was to determine a permeability coefficient ( $K_p$ ) and short-term absorption rate for vinyl toluene using human cadaver skin mounted in an *in vitro* diffusion cell model.

## MATERIALS AND METHODS

### A. Test Guidelines

The study design complies with the posting in the Federal Register April 26, 2004, Volume 69, Number 80, and the following test guidelines:

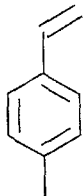
- OECD Guideline for the Testing of Chemicals. Draft New Guideline 428: Skin Absorption: *in vitro* Method. (2002)
- OECD Draft Guidance Document for the Conduct of Skin Absorption Studies. OECD Environmental Health and Safety Publications Series on Testing and Assessment No. 28. (2002)
- European Commission Guidance Document on Dermal Absorption. Sanco/222/2000 rev 6 (2002).

### B. Test Substance (CASN 25013-15-4)

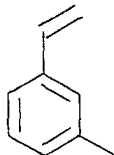
Vinyl toluene ( $\log P = 3.58$ ), a mixture of *para*-vinyl toluene (~40%) and *meta*-vinyl toluene (~60%) isomers, was supplied by the sponsor and assigned Haskell Laboratory Number 26622 upon receipt.

Structures:

*para*-vinyl toluene



*meta*-vinyl toluene



### C. Test System

#### 1. Human Skin

Samples of human cadaver skin from the National Disease Research Interchange (NDRI) were stored frozen at approximately -20°C until prepared for use. Samples were removed from donors and used within three months. Skin specimens selected for use were identified using a unique code (e.g., HCFA-26A = Human, Caucasian, Female, Abdomen sample 26-A).

#### 2. Justification for Selection of Test System

Dermal contamination is a potential route of human exposure. *In vitro* dermal techniques, which are required by the test rule described in the Federal Register dated April 26, 2004 (Volume 69, Number 80), have been shown to be a conservative model for predicting percutaneous absorption of various chemicals *in vivo*.<sup>(1-3)</sup>

#### 3. *In Vitro* Diffusion Cell Model

A static diffusion cell model was used for this study (Figure 1). The *in vitro* cells had an exposure area of 0.64 cm<sup>2</sup> and a receptor fluid chamber volume of approximately 5 mL.

### D. Dose Formulation and Concentration

The test substance was applied as received from the sponsor. The concentration of vinyl toluene applied to each skin replicate was based on the density of the test substance, which was 894.6 mg/mL (894,600 µg/cm<sup>3</sup>).

### E. Preparation of Skin Membranes

Samples of human cadaver skin obtained from the abdominal region, which were maintained frozen, were thawed at room temperature. Full thickness skin was immersed in 60°C water for 45 seconds to 2 minutes and the epidermis was peeled away from the dermis. The human

epidermal membrane was then placed onto an aluminum pan, with its identification written on the pan, and stored refrigerated at 0-10°C until readied for use. The thickness of representative membranes, as measured with a Mahr micrometer, ranged from 46 to 63  $\mu\text{m}$ .

#### **F. Membrane Equilibration and Assessment of Membrane Integrity**

Membranes were removed from refrigeration storage and hydrated in 0.9% saline for approximately 15 minutes. Following hydration, the membrane was mounted onto the top of the receptor chamber, *stratum corneum* uppermost, which was maintained with 0.9% saline. The donor chamber was then clamped in place and filled with 0.9% saline. The membrane was then allowed to equilibrate for approximately 30 minutes. During equilibration, the *in vitro* cells were heated using a recirculating water bath system to yield a receptor fluid temperature of 32°C. Following equilibration, the integrity of each membrane was assessed by measurement of electrical impedance prior to application of the test substance.<sup>(4-5)</sup>

Membranes with an impedance of  $\geq 17 \text{ k}\Omega$  were considered intact and retained for use on study. Saline in the donor and receptor chambers was removed prior to dosing, and the receptor chamber filled with fresh receptor fluid.

#### **G. Receptor Fluid**

The receptor chamber was filled with 0.9% saline containing 6% (w/v) polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether), and allowed to equilibrate for at least 15 minutes prior to dosing. The solubility of vinyl toluene in the selected receptor fluid was confirmed prior to study start.

#### **H. Exposure Groups, Target Parameters**

1. Determining the Permeability Coefficient ( $K_p$ )

##### Protocol Group: A

Number of skin replicates: 6, representing at least 3 donors

Dose volume: 100  $\mu\text{L}/\text{cm}^2$

Termination time: following steady-state determination

Following dose application, the donor chamber opening was occluded with Parafilm<sup>®</sup>, and serial receptor fluid samples were taken at 1, 2, 4, 8, 12, 24, 36, and 48 hours. The volume of receptor fluid in the receptor chamber was maintained by the replacement of a volume of fresh receptor fluid, equal to the sample volume. The receptor chamber arm remained occluded with Parafilm<sup>®</sup> at all times other than at sampling. At the end of the exposure period, the receptor fluid was removed and discarded.

2. Determining the Short-Term Absorption Rate, 10 and 60 minutes

Protocol Group: B

Number of skin replicates: 4, representing a single unique donor

Dose volume: 10  $\mu\text{L}/\text{cm}^2$

Termination times: 2 replicates at 10 minutes, 2 replicates at 60 minutes

Protocol Group: C

Number of skin replicates: 4, representing a single unique donor

Dose volume: 10  $\mu\text{L}/\text{cm}^2$

Termination times: 2 replicates at 10 minutes, 2 replicates at 60 minutes

Protocol Group: D

Number of skin replicates: 4, representing a single unique donor

Dose volume: 10  $\mu\text{L}/\text{cm}^2$

Termination times: 2 replicates at 10 minutes, 2 replicates at 60 minutes

Following dose application, the donor chamber opening was occluded with Parafilm<sup>®</sup>. At the end of the exposure period, the receptor fluid was removed and placed into a suitable container for analysis.

**I. Terminal Procedures**

At the conclusion of each exposure interval, the surface of each skin replicate was washed with a 2% Ivory Soap followed by rinsing with deionized (DI) water. The wash/rinse was discarded. The receptor and donors chambers were filled with 0.9% saline and an end of experiment electrical impedance measurement taken. Following the impedance measurement the saline in both the donor and receptor chambers was removed and discarded.

The skin membranes from the short-term exposure protocol groups B, C, and D were removed and placed into separate glass vials for extraction.

**J. Sample Handling and Processing**

The receptor fluid samples were not processed further. The skin samples from protocol groups B, C, and D were minced and extracted with methanol.

The concentration of vinyl toluene in the receptor fluid aliquots and the skin extract were determined using the following method and equipment.

System:	Agilent 1100 Series Equipment (Agilent Technologies, Palo Alto, CA, USA)
Column:	Zorbax RX-C8 2.1 mm x 150 mm, 5 $\mu$ m particles
Column temperature:	Ambient
Mobile phases:	A: Water, pH 2.8 (H <sub>3</sub> PO <sub>4</sub> ) B: Acetonitrile
Elution:	Isocratic, 50% (A), 50% (B)
Injection volume:	5 $\mu$ L
Flow rate:	0.5 mL/min
UV Wavelength:	254 nm

## K. Data Presentation

Vinyl toluene's permeability coefficient (Kp) was determined by plotting the amount of vinyl toluene detected in the receptor compartment at each serial collection time-point, adjusted for total receptor fluid volume, against time (in hours) to produce an absorption profile. Kp (i.e., cm/h) was calculated by dividing the penetration rate or slope of the line at steady-state (e.g.,  $\mu$ g vinyl toluene/cm<sup>2</sup>/h) by the concentration of applied chemical (894,600  $\mu$ g/cm<sup>3</sup>).

The short-term absorption rate ( $\mu$ g vinyl toluene/cm<sup>2</sup>/h) for each exposure interval (10 and 60 minutes) was calculated by dividing the sum of the vinyl toluene in the receptor fluid and skin by the skin exposure area (0.64 cm<sup>2</sup>) and exposure time.

Group data is presented as a mean  $\pm$  the standard deviation (SD) in the tables. Key observations of mean data are presented in the results section.

The values in the tables and appendices were generated by computer and rounded appropriately for inclusion in the report. As a consequence, calculations made using individual data in the appendices will, in some instances, yield a value that is not aesthetically the same.

## RESULTS AND DISCUSSION

### A. HPLC-UV Chromatography

(Figure 2)

Representative HPLC-UV chromatograms of a vinyl toluene analytical standard and vinyl toluene in receptor fluid are presented in Figure 2. Vinyl toluene was well resolved and gave the best response at a wavelength of 254 nm. Based on system response to vinyl toluene in undiluted receptor fluid, the limit of detection (LOD) and limit of quantitation (LOQ) was 0.16 and 0.54  $\mu$ g/mL, respectively. On average, method precision was 4.22 %CV.

### B. Solubility of Vinyl Toluene in Receptor Fluid

Vinyl toluene was determined to have a maximum solubility of 12,707  $\mu$ g/mL in 0.9% saline fortified with 6% (w/v) polyethoxyoleate (polyethylene glycol (PEG) 20 oleyl ether). This was approximately 143-fold higher than vinyl toluene's solubility in water alone ( $\sim$ 89  $\mu$ g/mL).

### C. Vinyl Toluene, Permeability Coefficient

(Tables 1-3, Figure 3, Appendix A)

Key observations of mean data:

- The integrity of human skin, as determined by electrical impedance (EI), was unaffected by continuous exposure, under occlusive conditions, to vinyl toluene; the ratio of the post-EI values to pre-EI values was 1.18. This slight increase in EI, which occurred over the exposure phase, may have been due to a raise in skin hydration.
- Vinyl toluene was detectable in the receptor fluid at the first serial sampling time-point (148.1  $\mu\text{g/mL}$ ). Based on the concentration-time course plot of time vs. vinyl toluene concentration ( $\mu\text{g/cm}^2$ ), steady state penetration was taken as the time interval 1 to 24 hours ( $n = 6$  data-points) with a slope 203.3  $\mu\text{g/cm}^2/\text{h}$ ; at the end of the 24-hour interval, <6% of the applied vinyl toluene was detected in the receptor chamber.
- Based on the slope at steady-state (203.3  $\mu\text{g/cm}^2/\text{h}$ ), and the concentration of the applied dose of vinyl toluene, taken as its density (894,600  $\mu\text{g/cm}^3$ ), the permeability coefficient was calculated to be  $2.27 \times 10^{-4}$  cm/h.

### D. Vinyl Toluene, 10 and 60 minutes Short-Term Absorption Rates

(Tables 4-5, Appendix B-C)

Key observations of mean data:

- The integrity of human skin, as determined by electrical impedance (EI), was unaffected by either short-term exposure interval of 10 and 60 minutes under occlusive conditions to vinyl toluene. The ratio of the post-EI values to pre-EI values for the 10-minute and 60-minute exposure groups were 1.27 and 1.12, respectively. As was observed for the skin from the permeability experiment, the EI increased slightly.
- Following a 10-minute exposure to a finite application of vinyl toluene, a total of 3.39  $\mu\text{g}$  of vinyl toluene was detected in the receptor fluid, with nearly an equivalent amount in the skin (3.79  $\mu\text{g}$ ). Based on the amount of vinyl toluene in the receptor fluid and skin (7.18  $\mu\text{g}$ ), an exposure area of 0.64  $\text{cm}^2$  and an exposure time of 0.17 hours, the short-term exposure rate was calculated to be 66  $\mu\text{g/cm}^2/\text{h}$ .
- Following a 60-minute exposure to a finite application of vinyl toluene, a total of 59.8  $\mu\text{g}$  of vinyl toluene was detected in the receptor fluid and 6.86  $\mu\text{g}$  in the skin. Based on the amount of vinyl toluene in the receptor fluid and skin (66.7  $\mu\text{g}$ ), an exposure area of 0.64  $\text{cm}^2$  and an exposure time of one hour, the short-term exposure rate was calculated to be 104.2  $\mu\text{g/cm}^2/\text{h}$ .

## CONCLUSIONS

Based on the slope at steady-state ( $203.3 \mu\text{g}/\text{cm}^2/\text{h}$ ) and the concentration of the applied dose of vinyl toluene, taken as its density ( $894,600 \mu\text{g}/\text{cm}^3$ ), the permeability coefficient was calculated to be  $2.27 \times 10^{-4} \text{ cm}/\text{h}$ .

Following a 10-minute exposure to a finite application of vinyl toluene, a total of  $3.39 \mu\text{g}$  of vinyl toluene was detected in the receptor fluid, with nearly an equivalent amount in the skin ( $3.79 \mu\text{g}$ ). Based on the amount of vinyl toluene in the receptor fluid and skin ( $7.18 \mu\text{g}$ ), an exposure area of  $0.64 \text{ cm}^2$  and an exposure time of 0.17 hours, the short-term exposure rate was calculated to be  $66 \mu\text{g}/\text{cm}^2/\text{h}$ .

Following a 60-minute exposure to a finite application of vinyl toluene, a total of  $59.8 \mu\text{g}$  of vinyl toluene was detected in the receptor fluid and  $6.86 \mu\text{g}$  in the skin. Based on the amount of vinyl toluene in the receptor fluid and skin ( $66.7 \mu\text{g}$ ), an exposure area of  $0.64 \text{ cm}^2$  and an exposure time of one hour, the short-term exposure rate was calculated to be  $104.2 \mu\text{g}/\text{cm}^2/\text{h}$ .

## RECORDS AND SAMPLE STORAGE

All data and analytical characterization records conducted by or for the sponsor will be retained by the sponsor. Raw data, and the final report will be retained at Haskell Laboratory, Newark, Delaware, or at Iron Mountain Records Management, Wilmington, Delaware, and will be returned to the sponsor within 6 months after the final report issues.

## REFERENCES

1. Scott, R.C., Batten, P.L., Clowes, H.M., Jones, B.K., and Ramsey, J.D. (1992). Further Validation of an *In Vitro* Method to Reduce the Need for *In Vivo* Studies for Measuring the Absorption of Chemicals through Rat Skin. *Fundamental and Applied Toxicology* 19, 484-492.
2. Ramsey, J.D., Woollen, B.H., Auton, T.R., and Scott, R.C. (1994). The Predictive Accuracy of *In Vitro* Measurements for Dermal Absorption of a Lipophilic Penetrant (Fluazifop-Butyl) through Rat and Human Skin. *Fundamental and Applied Toxicology* 23, 230-236.
3. Scott, R.C., Walker, M., and Dugard, P.H. (1986). A comparison of the *in vitro* permeability properties of human and some laboratory animal skins. *International Journal of Cosmetic Science* 8, 189-194.
4. Fasano, W.J., Manning, L.A., and Green, J.W. (2002). Rapid Integrity Assessment of Rat and Human Epidermal Membranes for *In Vitro* Dermal Regulatory Testing: Correlation of Electrical Resistance with Tritiated Permeability. *Toxicology In Vitro* 16, 731-740.
5. Fasano, W.J., Hinderliter, P.M. (2004). The Tinsley LCR Databridge Model 6401 and electrical impedance measurements to evaluate skin integrity *in vitro*. *Toxicology In Vitro* 18, 725-729.

**TABLES**

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TABLES

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EXPLANATORY NOTES

ABBREVIATIONS:

EI	electrical impedance
k-ohms	kilo-ohms
SD	standard deviation

**Table 1: Permeability coefficient, electrical impedance pre- and post- exposure**

Pre-EI (k-ohms)		Post-EI (k-ohms)		Ratio: Post/Pre	
Mean	SD	Mean	SD	Mean	SD
28.2	12.9	28.5	9.6	1.18	0.67

**Table 2: Permeability coefficient, vinyl toluene concentration-time course through human skin**

Time (hours)	Cumulative Amount Absorbed ( $\mu\text{g}$ vinyl toluene/ $\text{cm}^2$ )	
	Mean	SD
1	148.1	88.0
2	334.6	206.5
4	801.2	477.4
8	1717.4	955.6
12	2676.1	1683.6
24	4768.9	2792.2
36	15122.0	8041.4
48	22107.0	13702.6

**Table 3: Permeability coefficient, vinyl toluene absorption, steady-state penetration rate, and permeability coefficient**

Percent of Vinyl Toluene Absorbed at 24 Hours (%)		Steady-State Penetration Rate ( $\mu\text{g}/\text{cm}^2/\text{h}$ )		Permeability Coefficient (Kp) (cm/h)	
Mean	SD	Mean	SD	Mean	SD
5.33	3.12	203.3	120.0	$2.27 \times 10^{-4}$	$1.34 \times 10^{-4}$

**Table 4: Short-term absorption rates, pre-and post-electrical impedance**

Exposure Time	Pre-EI (k-ohms)		Post-EI (k-ohms)		Ratio: Post/Pre	
	Mean	SD	Mean	SD	Mean	SD
10 minute	24.5	4.2	30.7	8.3	1.27	0.34
60 minutes	28.5	4.2	31.5	7.7	1.12	0.32

**Table 5: Short-term absorption rates, receptor levels, skin levels, and penetration rates at 10 and 60 minutes**

Exposure Time	Receptor Fluid (RF) Vinyl Toluene (total µg)		Skin Vinyl Toluene (total µg)		Total Absorbed RF+Skin (total µg)		Penetration Rate (µg/cm <sup>2</sup> /h)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
10 minute	3.39	2.42	3.79	1.80	7.18	3.25	66.0	29.9
60 minutes	59.8	39.5	6.86	3.18	66.7	40.3	104.2	63.0

**FIGURES**

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FIGURES

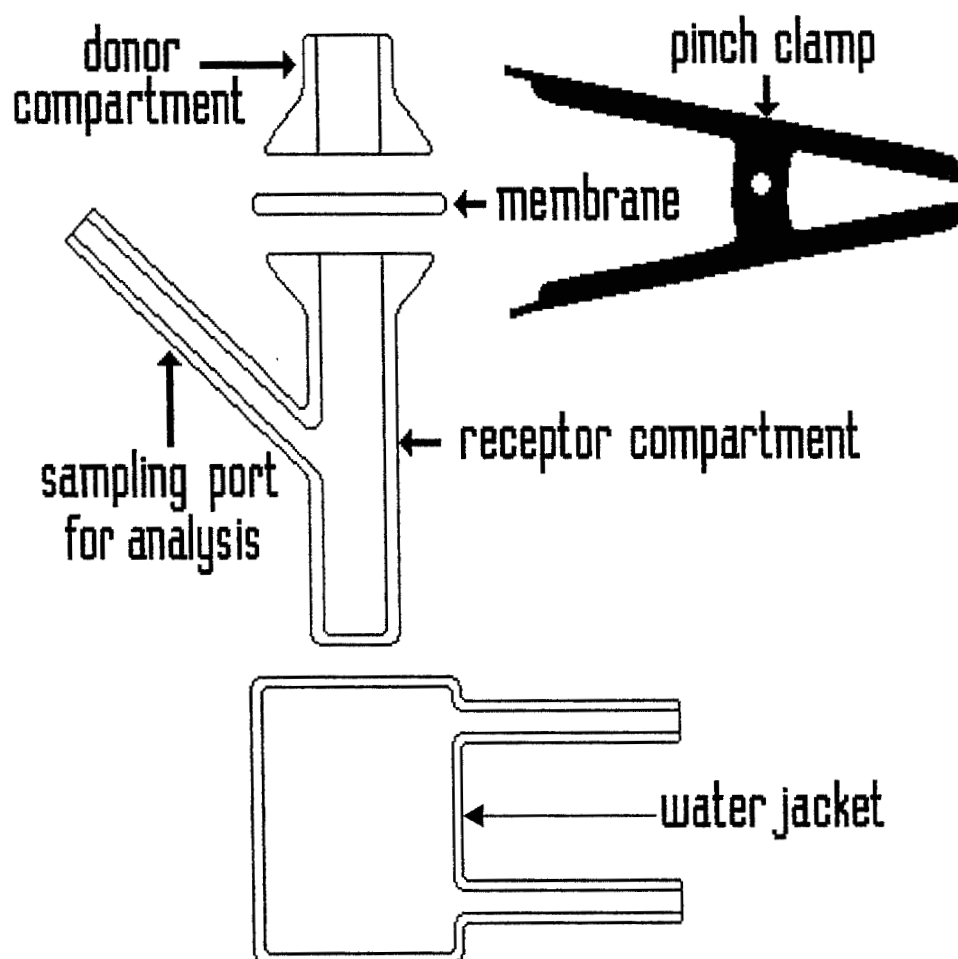
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EXPLANATORY NOTES

ABBREVIATIONS:

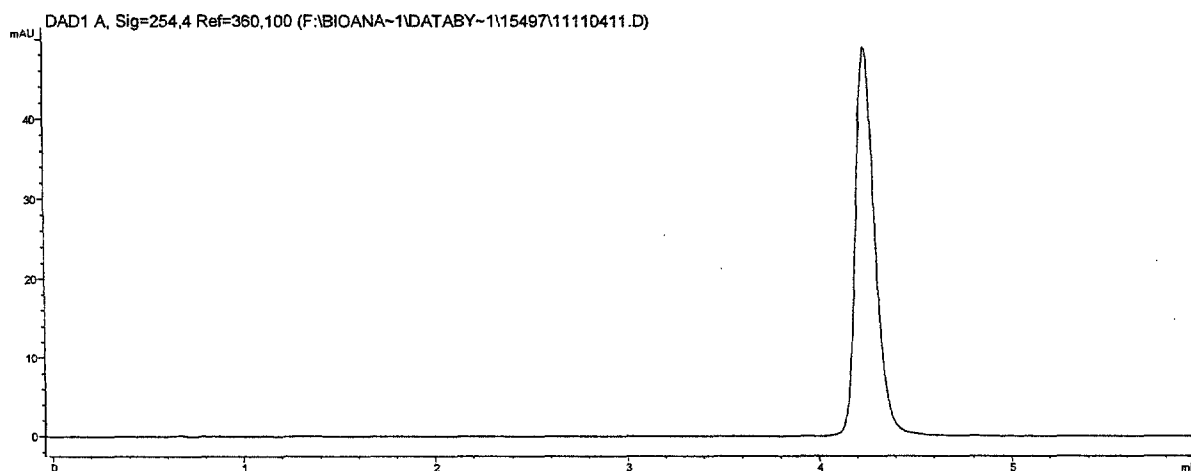
DAD	Diode Array Detector
h	hour(s)

**Figure 1: Static diffusion cell**

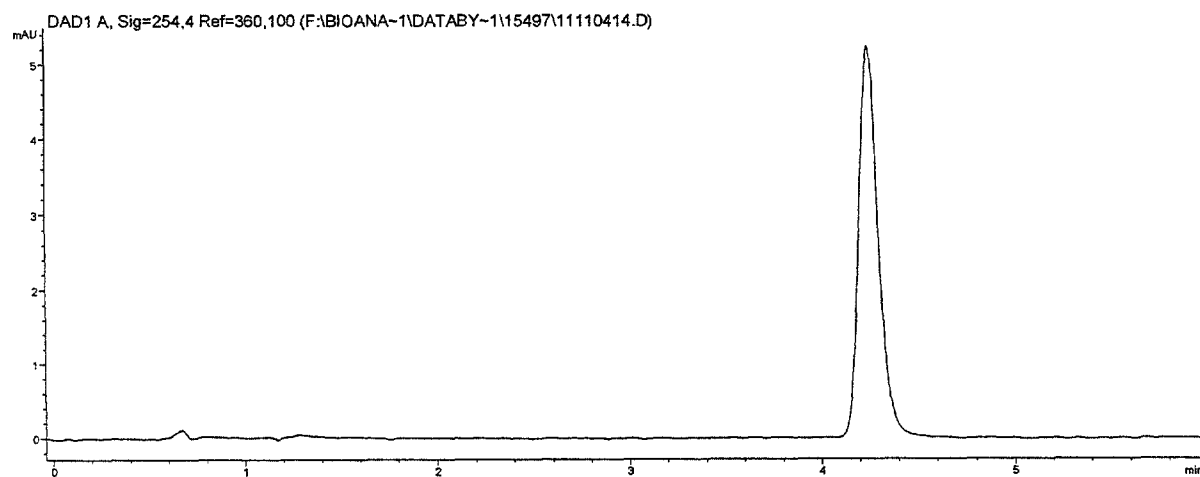


**Figure 2: Representative HPLC-UV chromatograms**

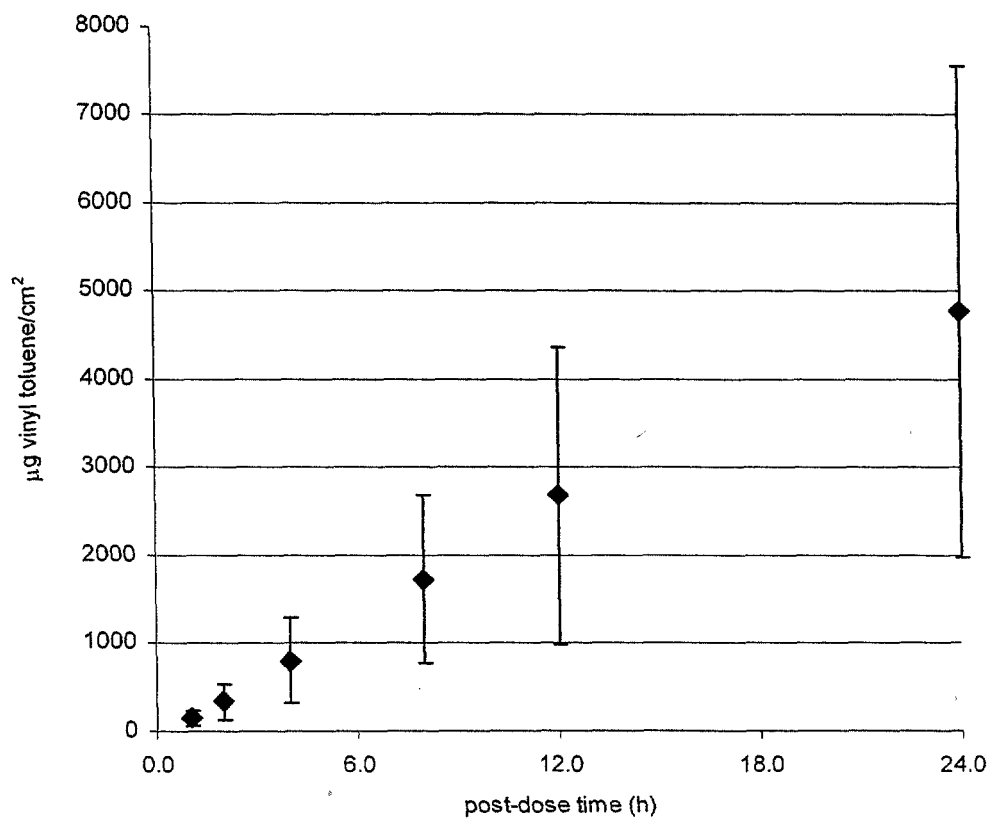
(a) Vinyl toluene analytical standard



(b) Vinyl toluene in receptor fluid



**Figure 3: Permeability coefficient, concentration-time course of vinyl toluene through human skin**



## APPENDICES

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APPENDICES

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**EXPLANATORY NOTES**

ABBREVIATIONS:

h	hour(s)
HCMA-80B	Human, Caucasian, Male, Abdomen, sample 80B
Kp	permeability coefficient
SD	standard deviation
VT	vinyl toluene

**Appendix A:**  
**Permeability Coefficient Data**

Pre-and post-electrical impedance

Cell ID	Skin ID	Pre-EI (k-ohms)	Post-EI (k-ohms)	Ratio: Post/Pre
A	HCMA-80B	28.4	18.9	0.67
B	HCMA-81	24.6	13.9	0.57
C	HCMA-82	17	32.7	1.92
D	HCFA-84	17	35.1	2.06
E	HCMA-80B	51.8	33.7	0.65
F	HCMA-81	30.4	36.7	1.21
Mean		28.2	28.5	1.18
SD		12.9	9.6	0.67

## Individual permeability data

WR-SC 15497-1623  
Protocol group A  
Formulation vinyl toluene (VT)  
Cell ID A  
Species Human  
Skin ID HCMA-80B

input

EI  
Pre 28.4  
Post 18.9

Volume of receptor fluid 4.9 mL  
Skin surface area 0.64 cm<sup>2</sup>  
Application volume rate 100 µL/cm<sup>2</sup>  
Application volume 64 µL  
Amount of VT applied 57254 µg  
Total VT applied per area 89460 µg/cm<sup>2</sup>

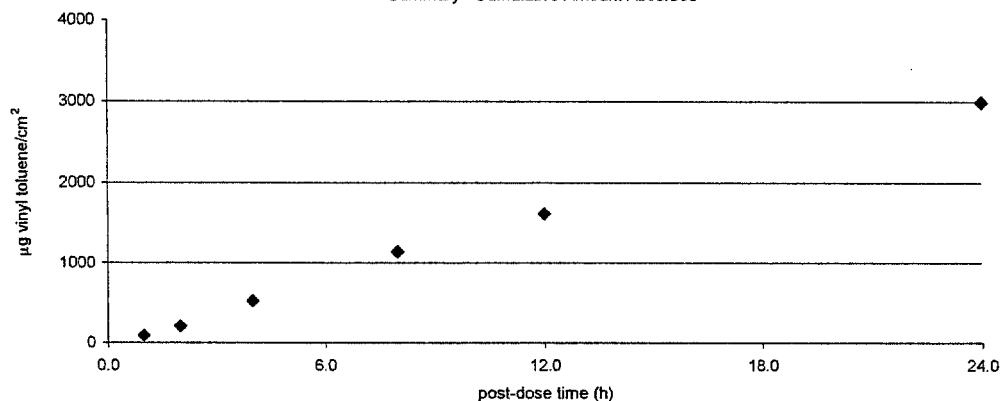
Sampling Occasion	1	2	3	4	5	6	7	8
Time after dosing (h)	1	2	4	8	12	24	36	48
Aliquot volume of receptor fluid (mL)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HPLC-UV, injection 1 (µg/mL)	11.6	26.2	65.6	140.0	190.9	354.8	1074.3	1379.8
HPLC-UV, injection 2 (µg/mL)	11.6	25.6	65.5	140.3	189.9	354.5	1076.1	1375.1
Average of injections (µg/mL)	11.6	25.9	65.6	140.2	190.4	354.7	1075.2	1377.5
Percent of maximum solubility	0.09	0.20	0.52	1.10	1.50	2.79	8.46	10.84
Amount of VT removed for interval (µg)	4.64	10.4	26.2	56.1	76.2	141.9	430.1	551.0
Cumulative amount of VT in receptor (µg)	56.8	131.6	336.2	728.0	1030.2	1911.2	5583.8	7494.9
Cumulative amount absorbed per area (µg/cm <sup>2</sup> )	88.8	205.5	525.3	1137.4	1609.8	2986.3	8724.7	11710.8
Cumulative percent absorbed	0.10	0.23	0.59	1.27	1.80	3.34	9.75	13.1

NB E-99681-BY

WR-SC 15497-1623  
Protocol group A  
Formulation vinyl toluene (VT)  
Cell ID A  
Species Human  
Skin ID HCMA-80B

NB E-99681-BY

Summary - Cumulative Amount Absorbed



Penetration rate at steady-state 126.5 µg/cm<sup>2</sup>/h  
Kp (permeability coefficient) 1.41E-04 cm/h  
Kp interval 1-24 hours  
correlation 0.998

Vinyl Toluene:  
In Vitro Dermal Absorption Rate Testing

DuPont-15743

WR-SC 15497-1623  
Protocol group A  
Formulation vinyl toluene (VT)  
Cell ID B  
Species Human  
Skin ID HCMA-81

input

EI  
Pre 24.6  
Post 13.9

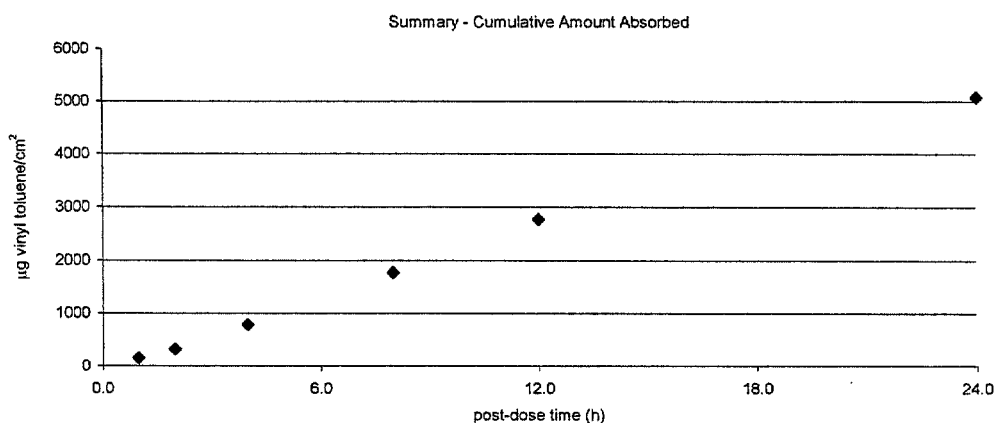
Volume of receptor fluid 4.8 mL  
Skin surface area 0.64 cm<sup>2</sup>  
Application volume rate 100 µL/cm<sup>2</sup>  
Application volume 64 µL  
Amount of VT applied 57254 µg  
Total VT applied per area 89460 µg/cm<sup>2</sup>

Sampling Occasion	1	2	3	4	5	6	7	8
Time after dosing (h)	1	2	4	8	12	24	36	48
Aliquot volume of receptor fluid (mL)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HPLC-UV, injection 1 (µg/mL)	19.3	40.5	101.6	223.1	337.2	617.2	2153.9	2929.0
HPLC-UV, injection 2 (µg/mL)	19.0	40.4	98.8	221.5	336.4	615.6	2158.0	2924.7
Average of injections (µg/mL)	19.2	40.5	100.2	222.3	336.8	616.4	2156.0	2926.9
Percent of maximum solubility	0.15	0.32	0.79	1.75	2.65	4.85	17.0	23.0
Amount of VT removed for interval (µg)	7.66	16.2	40.1	88.9	134.7	246.6	862.4	1170.7
Cumulative amount of VT in receptor (µg)	91.9	201.8	504.8	1131.0	1769.5	3246.3	10882.7	15445.4
Cumulative amount absorbed per area (µg/cm <sup>2</sup> )	143.6	315.3	788.8	1767.1	2764.8	5072.3	17004.2	24133.4
Cumulative percent absorbed	0.16	0.35	0.88	1.98	3.09	5.67	19.0	27.0

NB E-99681-BY

WR-SC 15497-1623  
Protocol group A  
Formulation vinyl toluene (VT)  
Cell ID B  
Species Human  
Skin ID HCMA-81

NB E-99681-BY



Penetration rate at steady-state 217.3 µg/cm<sup>2</sup>/h  
Kp (permeability coefficient) 2.43E-04 cm/h  
Kp interval 1-24 hours  
correlation 0.998

Vinyl Toluene:  
In Vitro Dermal Absorption Rate Testing

DuPont-15743

WR-SC 15497-1623  
Protocol group A  
Formulation vinyl toluene (VT)  
Cell ID C  
Species Human  
Skin ID HCMA-82

input

EI  
Pre 17.0  
Post 32.7

Volume of receptor fluid 5.0 mL  
Skin surface area 0.64 cm<sup>2</sup>  
Application volume rate 100 µL/cm<sup>2</sup>  
Application volume 64 µL  
Amount of VT applied 57254 µg  
Total VT applied per area 89460 µg/cm<sup>2</sup>

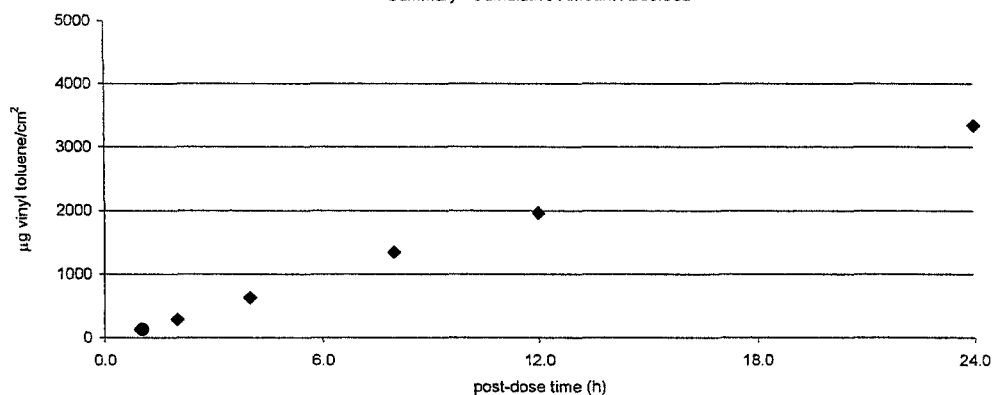
Sampling Occasion	1	2	3	4	5	6	7	8
Time after dosing (h)	1	2	4	8	12	24	36	48
Aliquot volume of receptor fluid (mL)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HPLC-UV, injection 1 (µg/mL)	16.6	34.5	76.7	162.4	227.5	392.1	1349.9	1889.1
HPLC-UV, injection 2 (µg/mL)	16.5	34.4	76.7	161.6	227.9	377.1	1351.5	1871.1
Average of injections (µg/mL)	16.6	34.5	76.7	162.0	227.7	384.6	1350.7	1880.1
Percent of maximum solubility	0.13	0.27	0.60	1.27	1.79	3.03	10.6	14.8
Amount of VT removed for interval (µg)	6.62	13.8	30.7	64.8	91.1	153.8	540.3	752.0
Cumulative amount of VT in receptor (µg)	82.8	178.9	403.9	861.1	1254.4	2130.0	7114.3	10301.6
Cumulative amount absorbed per area (µg/cm <sup>2</sup> )	129.3	279.5	631.1	1345.4	1960.0	3328.1	11116.1	16096.2
Cumulative percent absorbed	0.14	0.31	0.71	1.50	2.19	3.72	12.4	18.0

NB E-99681-BY

WR-SC 15497-1623  
Protocol group A  
Formulation vinyl toluene (VT)  
Cell ID C  
Species Human  
Skin ID HCMA-82

NB E-99681-BY

Summary - Cumulative Amount Absorbed



Penetration rate at steady-state 140.4 µg/cm<sup>2</sup>/h  
Kp (permeability coefficient) 1.57E-04 cm/h  
Kp interval 1-24 hours  
correlation 0.994

Vinyl Toluene:  
In Vitro Dermal Absorption Rate Testing

DuPont-15743

WR-SC 15497-1623

Protocol group A

Formulation vinyl toluene (VT)

Cell ID D input

Species Human

Skin ID HCFA-84

EI  
Pre 17.0  
Post 35.1

Volume of receptor fluid 4.8 mL  
Skin surface area 0.64 cm<sup>2</sup>  
Application volume rate 100 µL/cm<sup>2</sup>  
Application volume 64 µL  
Amount of VT applied 57254 µg  
Total VT applied per area 89460 µg/cm<sup>2</sup>

Sampling Occasion	1	2	3	4	5	6	7	8
Time after dosing (h)	1	2	4	8	12	24	36	48
Aliquot volume of receptor fluid (mL)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HPLC-UV, injection 1 (µg/mL)	42.6	95.7	221.5	446.8	728.5	1228.5	3775.9	5914.6
HPLC-UV, injection 2 (µg/mL)	42.4	95.3	220.2	445.8	726.9	1224.1	3788.0	5905.3
Average of injections (µg/mL)	42.5	95.5	220.9	446.3	727.7	1226.3	3782.0	5910.0
Percent of maximum solubility	0.33	0.75	1.74	3.51	5.73	9.65	29.8	46.5
Amount of VT removed for interval (µg)	17.0	38.2	88.3	178.5	291.1	490.5	1512.8	2364.0
Cumulative amount of VT in receptor (µg)	204.0	475.4	1115.3	2285.8	3815.0	6499.4	19257.0	30984.2
Cumulative amount absorbed per area (µg/cm <sup>2</sup> )	318.8	742.8	1742.6	3571.5	5961.0	10155.3	30089.1	48412.8
Cumulative percent absorbed	0.36	0.83	1.95	3.99	6.66	11.4	33.6	54.1

NB E-99681-BY

WR-SC 15497-1623

Protocol group A

Formulation vinyl toluene (VT)

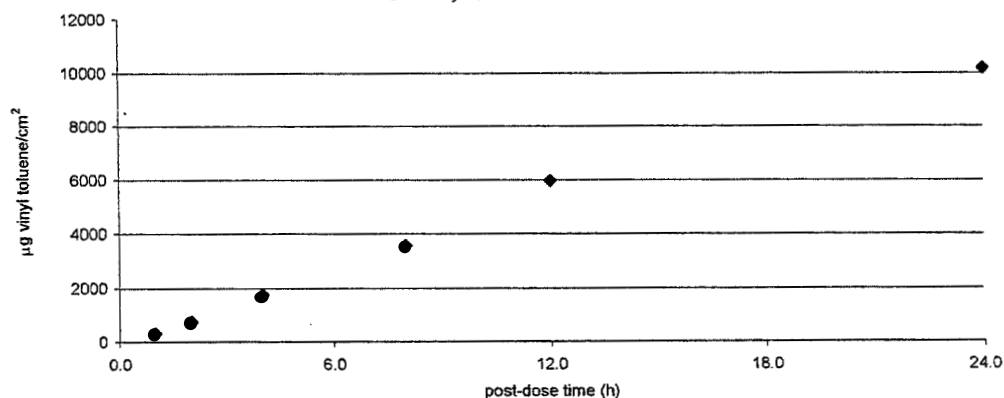
Cell ID D

Species Human

Skin ID HCFA-84

NB E-99681-BY

Summary - Cumulative Amount Absorbed



Penetration rate at steady-state

K<sub>p</sub> (permeability coefficient)

K<sub>p</sub> interval

correlation

434.5 µg/cm<sup>2</sup>/h  
4.86E-04 cm/h  
1-24 hours  
0.995

Vinyl Toluene:  
In Vitro Dermal Absorption Rate Testing

DuPont-15743

WR-SC 15497-1623  
Protocol group A  
Formulation vinyl toluene (VT)  
Cell ID E  
Species Human  
Skin ID HCMA-80B

input

EI  
Pre 51.8  
Post 33.7

Volume of receptor fluid 5.1 mL  
Skin surface area 0.64 cm<sup>2</sup>  
Application volume rate 100 µL/cm<sup>2</sup>  
Application volume 64 µL  
Amount of VT applied 57254 µg  
Total VT applied per area 89460 µg/cm<sup>2</sup>

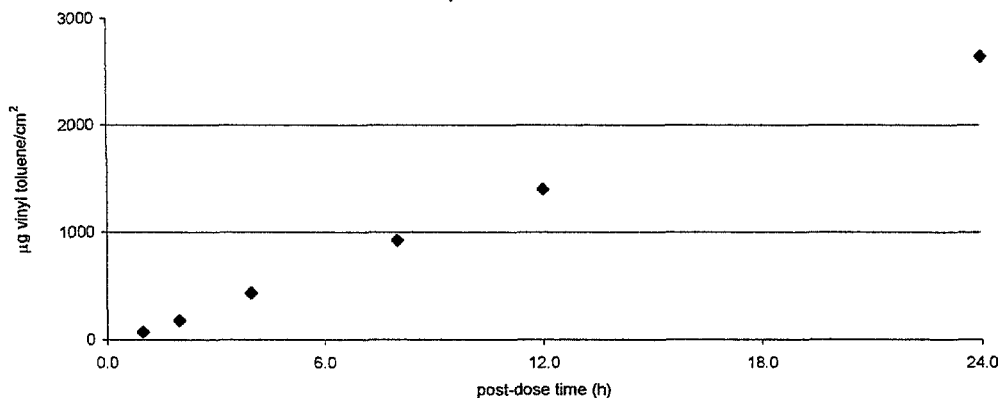
Sampling Occasion	1	2	3	4	5	6	7	8
Time after dosing (h)	1	2	4	8	12	24	36	48
Aliquot volume of receptor fluid (mL)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HPLC-UV, injection 1 (µg/mL)	9.3	21.8	52.1	110.0	160.4	304.6	1067.3	1431.2
HPLC-UV, injection 2 (µg/mL)	9.2	21.7	51.9	109.7	161.2	304.2	1062.7	1421.8
Average of injections (µg/mL)	9.25	21.8	52.0	109.9	160.8	304.4	1065.0	1426.5
Percent of maximum solubility	0.07	0.17	0.41	0.86	1.27	2.40	8.38	11.2
Amount of VT removed for interval (µg)	3.70	8.70	20.8	43.9	64.3	121.8	426.0	570.6
Cumulative amount of VT in receptor (µg)	47.2	114.6	277.6	593.4	897.2	1693.9	5694.7	7964.4
Cumulative amount absorbed per area (µg/cm <sup>2</sup> )	73.7	179.1	433.8	927.2	1401.9	2646.7	8898.0	12444.3
Cumulative percent absorbed	0.08	0.20	0.48	1.04	1.57	2.96	9.95	13.9

NB E-99681-BY

WR-SC 15497-1623  
Protocol group A  
Formulation vinyl toluene (VT)  
Cell ID E  
Species Human  
Skin ID HCMA-80B

NB E-99681-BY

Summary - Cumulative Amount Absorbed



Penetration rate at steady-state 112.5 µg/cm<sup>2</sup>/h  
Kp (permeability coefficient) 1.26E-04 cm/h  
Kp interval 1-24 hours  
correlation 0.999

Vinyl Toluene:  
In Vitro Dermal Absorption Rate Testing

DuPont-15743

WR-SC 15497-1623

Protocol group A

Formulation vinyl toluene (VT)

Cell ID F input

Species Human

Skin ID HCMA-81

EI  
Pre 30.4  
Post 36.7

Volume of receptor fluid

5.1 mL

Skin surface area

0.64 cm<sup>2</sup>

Application volume rate

100 µL/cm<sup>2</sup>

Application volume

64 µL

Amount of VT applied

57254 µg

Total VT applied per area

89460 µg/cm<sup>2</sup>

Sampling Occasion	1	2	3	4	5	6	7	8
Time after dosing (h)	1	2	4	8	12	24	36	48
Aliquot volume of receptor fluid (mL)	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
HPLC-UV, injection 1 (µg/mL)	16.9	34.6	82.2	184.9	271.7	510.0	1785.7	2267.0
HPLC-UV, injection 2 (µg/mL)	16.9	34.4	81.9	184.6	270.5	508.1	1781.6	2261.5
Average of injections (µg/mL)	16.9	34.5	82.1	184.8	271.1	509.1	1783.7	2264.3
Percent of maximum solubility	0.13	0.27	0.65	1.45	2.13	4.01	14.0	17.8
Amount of VT removed for interval (µg)	6.76	13.8	32.8	73.9	108.4	203.6	713.5	905.7
Cumulative amount of VT in receptor (µg)	86.2	182.7	439.0	995.6	1509.9	2831.9	9536.0	12700.5
Cumulative amount absorbed per area (µg/cm <sup>2</sup> )	134.7	285.5	686.0	1555.6	2359.2	4424.8	14899.9	19844.5
Cumulative percent absorbed	0.15	0.32	0.77	1.74	2.64	4.95	16.7	22.2

NB E-99681-BY

WR-SC 15497-1623

Protocol group A

Formulation vinyl toluene (VT)

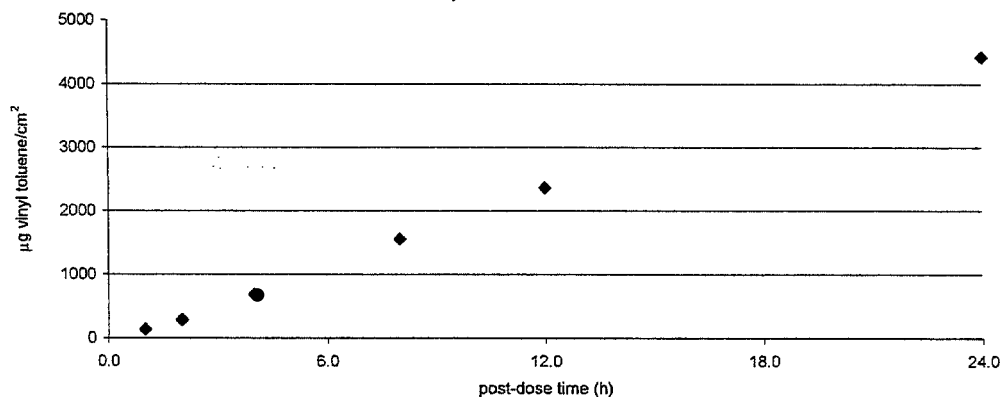
Cell ID F

Species Human

Skin ID HCMA-81

NB E-99681-BY

Summary - Cumulative Amount Absorbed



Penetration rate at steady-state

188.7 µg/cm<sup>2</sup>/h

Kp (permeability coefficient)

2.11E-04 cm/h

Kp interval

1-24 hours

correlation

0.999

Concentration-time course, cumulative amount of vinyl toluene in receptor fluid

Cumulative amount in receptor fluid ( $\mu\text{g}$  vinyl toluene/ $\text{cm}^2$ )

Cell ID	Time after dosing (hr)							
	1	2	4	8	12	24	36	48
A	88.8	205.5	525.3	1137.4	1609.8	2986.3	8724.7	11710.8
B	143.6	315.3	788.8	1767.1	2764.8	5072.3	17004.2	24133.4
C	129.3	279.5	631.1	1345.4	1960.0	3328.1	11116.1	16096.2
D	318.8	742.8	1742.6	3571.5	5961.0	10155.3	30089.1	48412.8
E	73.7	179.1	433.8	927.2	1401.9	2646.7	8898.0	12444.3
F	134.7	285.5	686.0	1555.6	2359.2	4424.8	14899.9	19844.5
Mean	148.1	334.6	801.2	1717.4	2676.1	4768.9	15122.0	22107.0
SD	88.0	206.5	477.4	955.6	1683.6	2792.2	8041.4	13702.6

Steady-state penetration, permeability coefficient

Cell ID	Percent Absorbed at 24 Hours	Penetration Rate $\mu\text{g}/\text{cm}^2/\text{h}$	Kp (cm/h)
A	3.34	126.5	$1.41 \times 10^{-4}$
B	5.67	217.3	$2.43 \times 10^{-4}$
C	3.72	140.4	$1.57 \times 10^{-4}$
D	11.35	434.5	$4.86 \times 10^{-4}$
E	2.96	112.5	$1.26 \times 10^{-4}$
F	4.95	188.7	$2.11 \times 10^{-4}$
Mean	5.33	203.3	$2.27 \times 10^{-4}$
SD	3.12	120.0	$1.34 \times 10^{-4}$

**Appendix B:**  
**10 Minute Exposure Data**

Pre-and post-electrical impedance

Exposure Time	Cell ID	Skin ID	Pre-EI (k-ohms)	Post-EI (k-ohms)	Ratio: Post/Pre
10 minutes	J	HCMA-80B	25.8	27.2	1.05
	K	HCMA-80B	20.8	24.3	1.17
	L	HCMA-81	29.9	39.1	1.31
	M	HCMA-81	27.4	40.1	1.46
	G	HCMA-82	18.6	33.5	1.80
	I	HCMA-82	24.2	19.7	0.81
		Mean	24.5	30.7	1.27
		SD	4.2	8.3	0.34

10 minute receptor fluid concentration data

Cell ID	Skin ID	Receptor fluid (RF) HPLC analysis	RF, HPLC ( $\mu\text{g VT/mL}$ )	Average RF ( $\mu\text{g VT/mL}$ )	Cell Volume (mL)	Total VT in RF ( $\mu\text{g}$ )
J	HCMA-80B	injection 1	0.56	0.570	4.8	2.74
		injection 2	0.58			
K	HCMA-80B	injection 1	0.059	0.069	5.0	0.35
		injection 2	0.079			
L	HCMA-81	injection 1	1.46	1.455	5.0	7.28
		injection 2	1.45			
M	HCMA-81	injection 1	0.86	0.860	5.1	4.39
		injection 2	0.86			
G	HCMA-82	injection 1	0.80	0.795	5.0	3.98
		injection 2	0.79			
I	HCMA-82	injection 1	0.32	0.320	5.1	1.63
		injection 2	0.32			
Mean						3.39
SD						2.42

10 minute skin concentration data

Cell ID	Skin ID	Skin HPLC analysis	Skin, HPLC ( $\mu\text{g VT/mL}$ )	Average HPLC ( $\mu\text{g VT/mL}$ )	Skin extraction (mL)	Total VT in Skin ( $\mu\text{g}$ )
J	HCMA-80B	injection 1	2.23	2.23	2.0	4.45
		injection 2	2.22			
K	HCMA-80B	injection 1	2.71	2.71	2.0	5.41
		injection 2	2.7			
L	HCMA-81	injection 1	2.95	2.95	2.0	5.90
		injection 2	2.95			
M	HCMA-81	injection 1	1.72	1.72	2.0	3.43
		injection 2	1.71			
G	HCMA-82	injection 1	0.64	0.64	2.0	1.28
		injection 2	0.64			
I	HCMA-82	injection 1	1.14	1.14	2.0	2.27
		injection 2	1.13			
Mean						3.79
SD						1.80

10 minute summary, total absorbed, penetration rate

Cell ID	Skin ID	VT in RF	VT in Skin	Total absorbed	Penetration rate
		( $\mu\text{g}$ )	( $\mu\text{g}$ )	RF+Skin ( $\mu\text{g}$ )	
J	HCMA-80B	2.74	4.45	7.19	66.0
K	HCMA-80B	0.35	5.41	5.76	52.9
L	HCMA-81	7.28	5.90	13.18	121.1
M	HCMA-81	4.39	3.43	7.82	71.8
G	HCMA-82	3.98	1.28	5.26	48.3
I	HCMA-82	1.63	2.27	3.90	35.9
Mean		3.39	3.79	7.18	66.0
SD		2.42	1.80	3.25	29.9

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**Appendix C:**  
**60 Minute Exposure Data**

Pre-and post-electrical impedance data

Exposure Time	Cell ID	Skin ID	Pre-EI (k-ohms)	Post-EI (k-ohms)	Ratio: Post/Pre
60 minutes	H	HCMA-80B	32.7	27.5	0.84
	I	HCMA-80B	31.4	30.2	0.96
	N	HCMA-81	30.8	42	1.36
	P	HCMA-81	29.1	35.6	1.22
	J	HCMA-82	25.5	19.6	0.77
	K	HCMA-82	21.6	34.2	1.58
		Mean	28.5	31.5	1.12
		SD	4.2	7.7	0.32

60 minute receptor fluid concentration data

Cell ID	Skin ID	Receptor fluid (RF) HPLC analysis	RF, HPLC ( $\mu\text{g VT/mL}$ )	Average RF ( $\mu\text{g VT/mL}$ )	Cell Volume (mL)	Total VT in RF ( $\mu\text{g}$ )
H	HCMA-80B	injection 1	8.15	8.12	5.1	41.41
		injection 2	8.09			
I	HCMA-80B	injection 1	2.99	2.97	5.1	15.12
		injection 2	2.94			
N	HCMA-81	injection 1	10.03	10.04	4.7	47.19
		injection 2	10.05			
P	HCMA-81	injection 1	27.04	26.99	4.9	132.25
		injection 2	26.94			
J	HCMA-82	injection 1	14.06	14.03	4.8	67.34
		injection 2	14.00			
K	HCMA-82	injection 1	11.15	11.14	5.0	55.70
		injection 2	11.13			
Mean						59.84
SD						39.54

60 minute skin concentration data

Cell ID	Skin ID	Skin HPLC analysis	Skin, HPLC ( $\mu\text{g VT/mL}$ )	Average HPLC ( $\mu\text{g VT/mL}$ )	Skin extraction (mL)	Total VT in Skin ( $\mu\text{g}$ )
H	HCMA-80B	injection 1	2.56	2.57	2.0	5.13
		injection 2	2.57			
I	HCMA-80B	injection 1	4.77	4.76	2.0	9.51
		injection 2	4.74			
N	HCMA-81	injection 1	3.96	3.96	2.0	7.91
		injection 2	3.95			
P	HCMA-81	injection 1	5.26	5.26	2.0	10.51
		injection 2	5.25			
J	HCMA-82	injection 1	0.89	0.89	2.0	1.78
		injection 2	0.89			
K	HCMA-82	injection 1	3.16	3.16	2.0	6.32
		injection 2	3.16			
Mean						6.86
SD						3.18

60 minute summary, total absorbed, penetration rate

Cell ID	Skin ID	VT in RF	VT in Skin	Total absorbed	Penetration rate
		( $\mu\text{g}$ )	( $\mu\text{g}$ )	RF+Skin ( $\mu\text{g}$ )	
H	HCMA-80B	41.4	5.13	46.5	72.7
I	HCMA-80B	15.1	9.51	24.6	38.5
N	HCMA-81	47.2	7.91	55.1	86.1
P	HCMA-81	132.3	10.51	142.8	223.1
J	HCMA-82	67.3	1.78	69.1	108.0
K	HCMA-82	55.7	6.32	62.0	96.9
Mean		59.8	6.86	66.7	104.2
SD		39.5	3.18	40.3	63.0